Symposium 16: Channels, Transporters, and Pores: Ways to Cross the Membrane

2785-Symp
Proton controlled transport systems: Experiments and Simulations
Isaiah T. Arkin.
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Protons, despite being the smallest ions in our cells, play key roles in numerous biological processes. The two systems that will be discussed exemplify this versatility: (i) The M2 protein from Influenza A is not only a H^+ channel but is gated by protons as well. Using linear and 2D FTIR spectroscopy, aided by noninvasive isotopic labeling, we were able to show the remarkable gating transition that takes place upon pH activation. Further studies that will be discussed include experimental and computational approaches that are being conducted to identify and understand the mode of activity of new anti-flu agents that block the M2 channel. (ii) The second system under investigation is the bacterial Na^+/H^+ antiporter. Here, motivated by our recent computational model (Science 2007, 317:799) we are expanding our studies using a comparative approach with antiporters from different bacteria. We will show both experimental and computational studies of these antiporters with the aim of enhancing our understanding of antiporter function. Specifically, we will address ion selectivity (K+ versus Na+) inhibitor binding and pH regulation.

2786-Symp
Structural Reorganisation of a K+ Channel Pore During Gating
Oliver B. Clarke, Alex Caputo, **Jacqui Gulbis**.
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While single channel recordings readily discern whether a K^+ pore is in a conducting or non-conducting state, the same does not apply to crystal structures of K^+ channels. There is at present no cogent means for demarcation of physiological state, or for pinpointing non-native structure arising when a channel is removed from the natural environment of a bilayer. The nature of molecular reconfiguration during gating remains indistinct (other than significant variability in width of the intracellular aperture). Defining a consistent set of molecular indicators of physiological state would lay a solid foundation for interpretation of K^+ channel structure, and enhance its usefulness to the biophysics community.
The architecture of the potassium selective pore is conserved in all known K^+ channels indicating that the core of the gating process may be universal, even though the means by which opening is instigated differs between families. We have targeted a single type of potassium channel for structure determination in conducting, intermediate, and non-conducting states. Our rationale is that excluding effects due to familial differences will pin down structural features reflecting the physiological gating transition. This initial study uses prokaryotic Kir channels, which are a good structural model for eukaryotic inward rectifiers. Our data suggest that discrete structural fingerprints characterise open and closed states.
An earlier study in collaboration with D.A. Doyle yielded the structure of a prokaryotic inward rectifier (KirBac1.1). Complete obstruction of the pore by phenylalanine side chains denoted an unambiguously non-conductive state, providing a structural reference point. A close homologue, KirBac3.1, has now yielded several further structures that collectively reveal a pattern of molecular rearrangement. A consistent sketch is emerging. Additionally, a reduction to 2-fold symmetry in some structures, particularly pronounced in the intracellular regions, indicates that Kir channel opening occurs stepwise.

2787-Symp
Protein Export Across the E. coli Outer Membrane by the Autotrantransporter EspP
Travis J. Barnard, Nathalie Dautin, Petra Lukacik, Harris D. Bernstein, **Susan K. Buchanan**.
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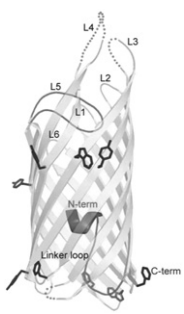
Autotransporters are outer membrane proteins produced by Gram-negative bacteria that are involved in virulence and consist of two domains, an N-terminal ''passenger domain'' and a C-terminal ''β-domain''. Passenger domains are secreted to the cell surface while β-domains are predicted to form β-barrel structures in the outer membrane. In some autotransporters, the secreted passenger domain is released from the β-domain by proteolytic cleavage. Using X-ray crystallography, we solved the 2.7 Å structure of the post-cleavage state of the β-domain of EspP, an autotransporter produced by *E. coli* O157:H7. The structure consists of a 12-stranded β-barrel with the passenger / β-domain cleavage junction located inside the barrel pore, approximately mid-way between the extracellular and periplasmic surfaces of the outer membrane. The structure reveals an unprecedented intra-barrel cleavage mechanism and suggests that two conformational changes occur in the β-domain after cleavage, one conferring increased stability on the β-domain and another restricting access to the barrel pore. The location of the cleavage site within the beta barrel has implications for secretion of the passenger domain across the outer membrane.



2788-Symp
Electron Microscopy of AQP0-mediated Membrane Junctions
Thomas Walz.
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Lens-specific aquaporin-0 (AQP0) functions as a specific water pore and forms the thin junctions between fiber cells in the lens. We describe a 1.9 Å resolution structure of junctional AQP0, determined by electron crystallography of double-layered two-dimensional crystals. Comparison of junctional and non-junctional AQP0 structures shows that junction formation depends on a conformational switch in an extracellular loop, which may result from cleavage of the cytoplasmic N- and C-termini. The pore in junctional AQP0 appears to be closed and retains only three water molecules in the center of the pore, which are too widely spaced to form hydrogen bonds with each other. Packing interactions between AQP0 tetramers in the crystalline array are mediated by lipid molecules, which assume preferred conformations. This made it possible to build an atomic model for the lipid bilayer surrounding the AQP0 tetramers and to describe non-specific lipid-protein interactions.

Symposium 17: The Dynamic Chromatin in Epigenetic Gene Control

2789-Symp
Mapping Epigenomes
Tarjei Mikkelsen.
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Next generation high-throughput sequencing instruments are emerging as powerful tools for comprehensive mapping of protein-DNA interactions and chromatin structure. I will describe ongoing efforts at the Broad Institute to create genome-wide maps of transcription factor binding, histone modifications and DNA methylation from a diverse set of human and mouse cell populations using high-throughput chromatin immunoprecipitation (ChIP-Seq) and bisulfite sequencing (HTBS). Our current studies are aimed at using these maps to catalog active, poised or repressed functional elements in each cell population, to elucidate the role of chromatin structure in the maintenance of epigenetic memory, and to identify sites of epigenetic dysregulation in malignancies. Initial comparisons of maps from cells in distinct lineages, and progressively differentiated cells in the same lineage, have yielded important insights into the potential and limitations of this approach.

2790-Symp
Structure And Chemistry Of The Human P300/CBP And Yeast Rtt109 Histone Acetyltransferases
Ronen Marmorstein.
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Histone actyltransferase (HAT) enzymes form a superfamily of proteins that transfer an acetyl group from the acetyl-coenzyme A (Ac-CoA) cofactor to the epsilon amino group of histone or sometimes non-histone proteins to promote gene activation. Paradoxally, despite the similar chemistry carried out by these enzymes, and their structurally related core regions, they fall into subfamilies with very limited to no sequence homology, structurally divergent core flanking regions and they use divergent mechanisms for catalysis. Four well studied HAT families include Gcn5/PCAF, MYST, p300/CBP and Rtt109. Although the structure and chemistry of the evolutionarily conserved Gcn5/PCAF and MYST HATs have been previously characterized, the metazoan-specific p300/CBP and fungal-specific Rtt109 have only recently been characterized at the structural and chemical levels. These recent studies provide new insights into the ancestral relationship between HATs and their functions and point to a common HAT ancestor that has evolved around a common structural framework to form HATs with divergent catalytic and substrate binding properties. These studies also point to the importance of regulatory loops within HATs and autoacetylation in HAT function. Implications for future studies will also be discussed.

2791-Symp
Chromatin Responses to DNA damage
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